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Pisolithus tinctorius Ectomycorrhizae Improve Survival and Growth of Pine Seedlings on Acid Coal Spoils in Kentucky and Virginia

DONALD H. MARX

Chief Plant Pathologist, Institute for Mycorrhizal Research and Development
USDA, Forest Service, Southeastern Forest Experiment Station
Forestry Sciences Laboratory, Carlton Street
Athens, Georgia 30602

JOEL D. ARTMAN

Assistant Chief, Insect and Disease Investigations Branch
Division of Forestry, Charlottesville, Virginia 29903

Nursery grown seedlings of loblolly (*Pinus taeda*) and shortleaf pine (*Pinus echinata*) with ectomycorrhizae formed by *Pisolithus tinctorius* survived and grew significantly better than seedlings with *Thelephora terrestris* ectomycorrhizae after 3 years on an acid coal spoil in Kentucky and 4 years on an acid coal spoil in Virginia. Seedlings with *Pisolithus* ectomycorrhizae on the Kentucky spoil had significantly more N and less S, Fe, Mn, and Al in needles than seedlings with *Thelephora* ectomycorrhizae after 3 years. Seedlings with *Thelephora* ectomycorrhizae were more susceptible to winter injury on both spoils. On the Kentucky spoil, slow-release nutrients from fertilizer tablets significantly increased seedling growth for the first 2 years, but after nutrients were depleted, seedlings exhibited N-deficiency symptoms. After 3 years, nonfertilized seedlings of *P. taeda* with *Pisolithus* ectomycorrhizae were larger than seedlings with *Thelephora* ectomycorrhizae and fertilizer tablets. The ability of *P. tinctorius* to persist and spread to new roots stimulated seedling growth on these acid spoils and the lack of persistence and spread of *T. terrestris* accounted for poor seedling survival and growth.

THE IMPORTANCE of ectomycorrhizae to the growth and development of pine in natural forest soils is well known. Many different species of fungi, mainly Basidiomycetes which produce mushrooms or puffballs, form ectomycorrhizae on roots of pine in the normal forest soil environment (Marks and Kozlowski, 1973). Certain of these fungi appear to be better adapted to extremes of soil conditions, such as pH, organic matter, moisture, and temperature, than are other fungal species (Trappe, 1977). Under stress conditions, some species of fungi survive and grow better on roots than others and, therefore, are more beneficial to their tree hosts. Many workers (Schramm, 1966; Medve et al., 1977; Marx, 1977a; Hile and Hennen, 1969; Meyer, 1968; Lampky and Peterson, 1963) have ob-

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served the widespread occurrence of sporophores of *Pisolithus tinctorius* under numerous tree species growing on acid coal spoils in various parts of the world. Usually only a few other species of ectomycorrhizal fungi occur under such adverse conditions. Marx (1977b) reported *Pisolithus* associated with trees growing on other such adverse sites, such as kaolin spoils and severely eroded sites in the southeastern United States. Pines need ectomycorrhizae to grow in natural situations and since *Pisolithus* is commonly associated with pines growing on acid coal spoils and other adverse sites, it is logical to assume that *Pisolithus* ectomycorrhizae contribute significantly to the health and vigor of seedlings on these sites.

Nursery grown pine seedlings are used in many reclamation programs in the eastern United States. Following fumigation of nursery soils, seedlings become ectomycorrhizal with *Thelephora terrestris*, a fungus particularly adaptable to the high fertility and moisture normally found in nurseries. It is spread naturally via windblown spores. Pine seedlings with *Thelephora* ectomycorrhizae do not survive or grow well at high soil temperatures encountered during summer months on coal spoils; seedlings with *Pisolithus* ectomycorrhizae, however, survive and grow well at high soil temperatures (Schramm, 1966; Marx, Bryan and Davey, 1970; Marx and Bryan, 1971). The generally poor performance of nursery seedlings after outplanting on many coal spoils may be partially due to the fact that the fungal symbiont on their roots at planting cannot tolerate the adversity of the coal spoil. Seedlings that do survive usually begin to grow rapidly following the natural but erratic appearance of *Pisolithus* ectomycorrhizae on root systems (Schramm, 1966; Marx 1977a). *Pisolithus* occurs at random from windblown basidiospores released from sporophores produced in adjacent forests. The above observations indicate that survival and growth of pine seedlings on acid coal spoils may be improved if *Pisolithus* ectomycorrhizae were formed on the seedlings in the nursery prior to their outplanting on the spoils.

Experimental procedures have been developed to artificially infest fumigated nursery soils with pure cultures of *P. tinctorius* in order to form abundant *Pisolithus* ectomycorrhizae on pine seedlings (Marx and Bryan, 1975; Marx, Bryan and Cordell, 1976; Marx and Artman, 1978; Marx, Morris and Mexal, 1978). Two-year field tests on routine reforestation sites in North Carolina and Florida revealed that pine seedlings with abundant *Pisolithus* ectomycorrhizae survived and grew better than seedlings with fewer *Pisolithus* ectomycorrhizae, or seedlings having only *Thelephora* ectomycorrhizae. Growth differences of 25 to 450 percent were observed on different pine species in certain of these comparisons (Marx, Bryan and Cordell, 1977). It became apparent from field studies that as site quality decreased, the value of *Pisolithus* ectomycorrhizae to the seedlings increased. *Pisolithus* maintained its dominance on seedling roots on the poorer sites but other ectomycorrhizal fungi were also frequently observed on seedlings on the better quality sites after 2 years. In other tests on the severely eroded sections of the Copper Basin of Tennessee, seedlings of *Pinus virginiana* and *P. taeda* with *Pisolithus* ectomycorrhizae were nearly twice as large after two years as seedlings with only *Thelephora* ectomycorrhizae (Berry and Marx, 1978). Both fungi persisted on seedling roots very well on this site, apparently because few other competitive ectomycorrhizal fungi were present.

The studies reported here were designed to determine the significance of *P. tinctorius* ectomycorrhizae to survival and growth of pines on coal spoils in Kentucky and Virginia. Since several workers (Zarger et al., 1973) have shown the value of fertilizer to growth of trees on coal spoils, a fertility variable was also tested on the Kentucky spoil. Slow release fertilizer, in the form of seedling starter-tablets, was used because they have been shown to benefit early growth of tree seedlings on borrow pits (White, 1963) and routine reforestation sites (Austin and Strand, 1960; Meskimen, 1971).

MATERIALS AND METHODS

Kentucky Coal Spoil

Seedling Production: Mycelial inoculum of *P. tinctorius* (isolate 126) was grown for 3 months in 2 liter containers and leached (Marx and Bryan, 1975). The inoculum was broadcast at a rate of 1.6 l/m^2 of soil surface with 560 kg/ha of 10- 10- 10 fertilizer onto recently fumigated nursery soil and mechanically incorporated 12 cm into the soil. Autoclaved inoculum was similarly prepared and added to soil for production of control seedlings which became ectomycorrhizal with naturally occurring *T. terrestris*. The soil, a 2:1:1 volume mixture of forest clay loam: sand: milled pine bark, contained in 3 m×1 m×0.3 m deep, wood-frame plots was fumigated with recommended rates of methyl bromide. Seeds of *P.*

taeda (North Carolina seed source) and *P. echinata* (northern Georgia seed source) were coated with Arasan® and late sticker and planted in April 1974 in rows 10 cm apart in the plots. Seed of each pine species were planted in one inoculated and one noninoculated (control) plot. Six weeks after seed germination, seedlings in all plots were thinned to a density of 270 per m^2. Seedlings were watered as needed during the growing season. In late June, all plots were fertilized with 56 kg N/ha as $NH_4 NO_3$.

In February 1974, roots of all seedlings were cut vertically between rows and undercut at a depth of 20 cm with a flat shovel. Seedlings were lifted by hand and graded. Loblolly and shortleaf pine seedlings were graded to heights of 20 and 15 cm (± 10%) and root collar diameters of 0.3 and 0.4 cm, respectively, for both ectomycorrhizal treatments. Seedlings with less than 70 to 80 percent of their feeder roots in an ectomycorrhizal condition were discarded. Control seedlings had the majority of their ectomycorrhizae formed by *T. terrestris* and seedlings from soil infested with *P. tinctorius* had at least 90 percent of their total ectomycorrhizae formed by *Pisolithus;* the remainder were formed by *Thelephora*. Ectomycorrhizal assessments were done visually (Marx and Bryan, 1975). Seedlings were stored in moist peat moss in plastic-lined, kraft paper seedling bags at 5°C for 1 month prior to planting.

Study Installation: The planting site was located on a mountain top approximately 32 km north of London, Kentucky. The entire mountain top was strip-mined in 1971 leaving a spoil of approximately 8 ha. The spoil was planted in the fall of 1972 and again in 1973 with loblolly pine seedlings produced in a conventional tree nursery in Kentucky. Less than 10 percent of these seedlings survived; those living were severely stunted. The survivors were removed from the area prior to installation of this experiment.

Five blocks, each with eight 5×5 m plots, were installed on the spoil. A 3 m wide nonplanted border separated each plot. One of eight treatments was randomly placed in each of the 8 plots per block. The treatments in the random block design were loblolly or shortleaf pine seedlings with either *Pisolithus* or *Thelephora* ectomycorrhizae planted with or without a fertilizer tablet. One tablet (21 gm, 20-10-5 formulation, Sierra Chemical Co., Newark, CA 94560) per seedling was placed in the closing hole approximately 8 cm deep and 8 cm from the seedling roots. Twenty-five seedlings were planted by hand in each plot in 5 rows of 5 seedlings each. Spacing of seedlings between and within rows was approximately 1.25 m. Several spoil samples were removed to a depth of 0 to 15 cm from each plot, combined into one composite sample per plot and chemically analyzed.

Seedling Data: Two weeks after planting, height and root collar diameters were obtained from each seedling to confirm that seedlings of both pine species, regardless of initial ectomycorrhizal treatment, were of comparable size at planting. This was done to eliminate any possiblility of interaction between seedling size and field performance (Wakeley, 1954).

Measurements of survival, height and root collar diameters were obtained in November 1975, October 1976, and December 1977. Also, at each measurement period incidence of sporophores of *P. tinctorius* and *T. terrestris* were recorded and roots were removed from 3 random seedlings per plot and examined for specific ectomycorrhizae and hyphal strands of fungi. In 1976 and 1977, current-year needle samples were removed from 3 seedlings per plot and chemically analyzed for major and minor elements using routine procedures.

Survival and growth data after each growing season were integrated into indices of plot volume. Height × (root collar diameter)^2 was considered to be an indicator of seedling volume. Plot volume index (PVI) was determined by multiplying mean seedling volume by the number of surviving seedlings per plot (Marx et al., 1976). Analyses of variance were made on all data and differences among means were evaluated with Duncan's New Multiple Range Test (P=0.05).

Virginia Coal Spoil

Seedling Production: Loblolly pine seedlings (Virginia seed source) with *Pisolithus* (isolate 138) or *Thelephora* ectomycorrhizae outplanted on the Virginia coal spoil were grown under the same conditions used to produce seedlings for the Kentucky coal spoil. These seedlings were grown, however, from April 1973 to March 1974, at which time they were lifted. Unfortunately, while in the nursery a warm period occurred in January causing many seedlings to break dormancy and grow vigorously. Many were killed by subsequent frosts, reducing the number of seedlings available for outplanting. Because of this occurrence and resulting variables in seedling size the degree of ectomycorrhizal development was the main criteria for grading. Graded seedlings from the *Pisolithus* plot had a total ectomycorrhizal development of 85 percent of

which about 90 percent were formed by *Pisolithus*; the remainder was formed by *Thelephora*. Seedlings from the noninoculated, control plot had a total ectomycorrhizal development of about 80 percent, all of which were formed by naturally occurring *Thelephora*. Seedlings were packaged, as described earlier, shipped to Virginia, and planted within two days of their arrival.

Study Installation: The site is located in Buchanan County in southwest Virginia. The area was strip-mined several years previously and was selected for the study due to high soil temperature and unusually low pH. The area had been unsuccessfully seeded with a grass mixture earlier. Six paired plots were placed at random on level areas on the spoil. Each plot was 6.1×6.1 m and was separated from its companion plot by a 7 m wide nonplanted area. Seedlings with either *Pisolithus* or *Thelephora* ectomycorrhizae were planted in a randomly selected plot of each pair. Twenty-five seedlings were planted by hand in each plot in 5 rows of 5 seedlings each. Spacing of seedlings between and within rows was 1.5 m. Spoil samples were removed from a depth of 0 to 15 cm from three random plots and analyzed for pH, organic matter, and nitrate-N.

Seedling Data: One month after planting, height measurements were obtained to determine possible differences in seedling size caused by ectomycorrhizal treatment and lack of size-grading at the nursery.

Measurements of survival, height and root collar diameters were obtained in October 1974, December 1975, March 1977, and December 1977. Incidence of sporophores was recorded and an evaluation of seedling root systems was done at these times using procedures described earlier. Unfortunately, vandals uprooted and removed over 10 percent of the test seedlings just prior to obtaining 2nd year measurements in December 1975. Second year survival data were obtained by counting the holes as surviving trees. Survival data of subsequent years were based on existing trees. Because of the vandalism, the plot volume index parameter was not used to compare treatment effects in this study. All data were statistically analyzed using the paired t-test at P=0.05.

RESULTS

Kentucky Coal Spoil

Chemical analyses of spoil samples collected at planting revealed that, by random chance, seedlings with *Thelephora* ectomycorrhizae were planted in plots that were significantly less acid, had less total Fe, but more available P and Ca than plots planted to *Pisolithus* seedlings. The pH of the spoil in the loblolly pine plots was 4.1 in the *Pisolithus* plots and 4.6 in the *Thelephora* plots; in the shortleaf pine plots, the reactions were pH 4.1 and 4.3, respectively. Phosphorus levels were 3 and 7 p/m in the *Pisolithus* and *Thelephora* plots, respectively, for loblolly pine and 2 and 6 p/m for shortleaf pine, respectively. Calcium varied from 467 p/m in the *Pisolithus* plots to 1241 p/m in the *Thelephora* plots of loblolly pine and 483 p/m to 691 p/m for shortleaf pine of *Pisolithus* and *Thelephora*. Total Fe in the corresponding loblolly and shortleaf pine plots was 35 and 34 p/m for *Pisolithus* and 46 and 49 p/m for *Thelephora* plots, respectively. Amounts of K (47 to 52 p/m), Mg (81 to 145 p/m), Mn (12 to 27 p/m), Zn (5 to 10 p/m), and Al (4 to 6 p/m) did not vary significantly. Statistical comparison of seedlings measurements obtained 2 weeks after planting revealed that all seedlings of a pine species, regardless of initial ectomycorrhizal treatment, had the same height and root collar diameters.

Survival and growth of loblolly pine were significantly influenced by ectomycorrhizae and the fertilizer tablets (Table 1). After the first growing season, there was no difference in survival or height growth of loblolly pine seedlings due to the treatment but seedlings with *Thelephora* ectomycorrhizae without the fertilizer tablet had smaller root collar diameters than other seedlings. However, differences became more apparent after these parameters were calculated to plot volume indices (PVI). After the first year, seedlings with *Pisolithus* ectomycorrhizae and the tablet had significantly larger PVI than seedlings with *Thelephora* ectomycorrhizae either with or without the fertilizer tablet. Growth differences due to *Pisolithus* ectomycorrhizae were even greater after the 2nd and 3rd growing seasons. The effect of the fertilizer tablet was highly significant after the 2nd growing season, but by the end of the 3rd year seedlings with *Pisolithus* ectomycorrhizae only were as large as seedlings with the tablet? added

The fertilizer tablet increased the PVI of seedlings with *Thelephora* ectomycorrhizae after 2 and 3 years. The severe winter of 1976-77 caused a significant degree of mortality among seedlings with *Thelephora* ectomycorrhizae after the 3rd year. The spoil froze to a depth of approximately 50 cm, which was accompanied by persistent dry and cold winds for several weeks. By early

Table 1. Survival and Growth of Loblolly and Shortleaf Pine Seedlings with Pisolithus or Thelephora Ectomycorrhizae and With or Without a Fertilizer "Starter" Tablet After 1, 2, and 3 Years on a Coal Spoil in Kentucky

Year	Treatment	Percent Survival	Height (m)	Root Collar Dia (cm)	PVI* (cm^3) × 10^2
			---------- Loblolly pine ----------		
1	*Pisolithus* with tablet	89a	0.41a	1.03a	11a
	Pisolithus without tablet	84a	0.38a	0.91a	7ab
	Thelephora with tablet	80a	0.39a	0.83ab	5b
	Thelephora without tablet	90a	0.30a	0.55b	2b
2	*Pisolithus* with tablet	89a	1.07a	2.71a	196a
	Pisolithus without tablet	84a	0.95a	2.52a	128b
	Thelephora with tablet	80a	0.84a	1.83ab	57c
	Thelephora without tablet	87a	0.55b	1.26b	22d
3	*Pisolithus* with tablet	80a	1.45a	3.69a	348a
	Pisolithus without tablet	80a	1.45a	3.84a	351a
	Thelephora with tablet	68b	1.06b	2.53ab	102b
	Thelephora without tablet	75ab	0.83b	2.15b	68c
			---------- Shortleaf pine ----------		
1	*Pisolithus* with tablet	71a	0.30a	0.93a	5a
	Pisolithus without tablet	66a	0.23ab	0.65ab	2b
	Thelephora with tablet	71a	0.24ab	0.67ab	2b
	Thelephora without tablet	69a	0.19b	0.48b	1b
2	*Pisolithus* with tablet	69a	0.79a	2.37a	84a
	Pisolithus without tablet	66a	0.55ab	1.64ab	36b
	Thelephora with tablet	70a	0.62ab	1.78ab	53b
	Thelephora without tablet	63a	0.41b	1.15b	9c
3	*Pisolithus* with tablet	69a	1.26a	3.52a	237a
	Pisolithus without tablet	64a	1.06b	3.08b	136b
	Thelephora with tablet	65a	0.84bc	2.34c	114b
	Thelephora without tablet	61a	0.65c	1.82d	27c

* PVI (Plot Volume Index), cm^3=(root collar diameter, cm)^2 × height, cm × number surviving seedlings per plot.

Means within a given year and tree species, but between treatments with a common letter are not significantly different at P=0.05.

March 1977, about 75 percent of needles of all seedlings in each treatment were brown. Seedlings with *Pisolithus* ectomycorrhizae, however, survived better, formed new needles, and initiated height-growth at least 6 weeks earlier than seedlings with *Thelephora* ectomycorrhizae. More seedlings with *Thelephora* ectomycorrhizae and the fertilizer tablet died than from any other treatment. By the end of the 3rd year, loblolly pine seedlings with *Pisolithus* ectomycorrhizae, independent of fertilizer tablets, had 12 percent better survival, were 53 percent taller, and had 61 percent larger root collar diameters than seedlings with *Thelephora* ectomycorrhizae. These increases resulted in an increase of more than 310 percent in PVI. Independent of ectomycorrhizae, the fertilizer tablets decreased survival by 5 percent, increased height by 10 percent, and increased root collar diameter by 4 percent after the 3rd year. These parameters increased the PVI by only 7 percent.

Survival of shortleaf pine seedlings was not affected by treatment at any time during the period including the winter of 1976-77 (Table 1). On the basis of PVI, *Pisolithus* ectomycorrhizae and the fertilizer tablets improved seedling growth as early as the 1st year in comparison to seedlings with only *Thelephora* ectomycorrhizae. By the end of the 3rd year, seedlings with *Pisolithus* ectomycorrhizae and the fertilizer tablet still outperformed others. Seedlings with *Pisolithus* ectomycorrhizae only were as large as seedlings with *Thelephora* ectomycorrhizae and the fertilizer tablet. As a group, shortleaf pine seedlings with *Pisolithus* ectomycorrhizae had 6 percent better survival, 56 percent greater height

Table 2. Concentration of Elements in Needles Sampled After 2 and 3 Years From Loblolly and Shortleaf Pine Seedlings Planted on a Coal Spoil in Kentucky as Affected** by Pisolithus or Thelephora Ectomycorrhizae Interacting with Fertilizer "Starter" Tablets. Each Value is the Mean of Combined Samples from Three Random Seedlings from Each of Five Plots per Treatment Each Year*

Treatment	Sample Year	%			P/m		
		N	Ca	S	Fe	Mn	Al
				Loblolly pine			
Pisolithus with fertilizer tablet	2	1.19b	.214ab	.131b	60b	656b	622b
	3	1.12b	.257a	.112ab	—	912b	877b
Pisolithus without fertilizer tablet	2	1.67a	.152b	.146b	58b	826ab	543b
	3	1.38a	.220a	.106b	—	986b	822b
Thelephora with fertilizer tablet	2	0.96b	.247a	.156b	72ab	1139a	784ab
	3	1.04b	.291a	.128a	—	1224a	1059a
Thelephora without fertilizer tablet	2	1.16b	.258a	.218a	92a	1116a	755a
	3	1.13b	.262a	.126a	—	1180a	909ab
				Shortleaf pine			
Pisolithus with fertilizer tablet	2	1.43ab	.195a	.146b	97b	627a	829ab
	3	1.24a	.214a	.108b	—	790b	913b
Pisolithus without fertilizer tablet	2	1.70a	.166a	.149b	140ab	682a	741b
	3	1.29a	.192a	.120ab	—	800b	869b
Thelephora with fertilizer tablet	2	1.27b	.203a	.201ab	105b	860ab	977ab
	3	1.02b	.254a	.122ab	—	984a	1285a
Thelephora without fertilizer tablet	2	1.41ab	.179a	.242a	214a	1067a	1157a
	3	1.31a	.246a	.148a	—	1010a	1278a

*P, K, Mg, and Zn were not significantly affected by the treatments.
**Means within a given year and tree species, but between treatments with a common letter are not significantly different at P=0.05.

growth and 59 percent larger root collar diameters in comparison to seedlings with *Thelephora* ectomycorrhizae. This resulted in a 165 percent greater PVI by the end of the 3rd year. Independent of the ectomycorrhizae, the fertilizer tablets at the end of the 3rd year increased survival by 7 percent, height by 23 percent and root collar diameters by 20 percent. This resulted in a 115 percent increase in PVI. These data suggest that shortleaf pine was more nutrient demanding than loblolly pine on this site.

Pisolithus ectomycorrhizae were found to totally dominate the root systems of both pine species with or without the fertilizer tablet in all *Pisolithus* plots. Few nonmycorrhizal roots were detected on any of these seedlings. By the end of the 2nd year, yellow-gold hyphal strands of this fungus were evident to the unaided eye in the upper 10 cm of spoil as far as 1.5 m from the closest seedling. Sporophore production by *P. tinctorius* was also abundant. After the 3rd year, loblolly pine with and without the fertilizer tablet had a yearly production of 2.6 and 2.1 sporophores/m^2 of spoil surface, respectively; shortleaf pine had 2.1 and 1.8 sporophores/m^2 of spoil surface, respectively. Sporophores varied in size from 2 to 18 cm in diameter; most had stalks from 7 to 12 cm in height.

Root systems of seedlings of both pine species with *Thelephora* ectomycorrhizae, with and without fertilizer tablets, had a low incidence of *Thelephora* ectomycorrhizae. Less than half of the new lateral roots (those extending from the original root system) produced by the end of the 3rd year had visually identifiable ectomycorrhizae. Many young, nonmycorrhizal feeder roots were dark in color and appeared to be nonfunctional. Only a trace (less than 1 percent) of *Pisolithus* ectomycorrhizae were observed on these seedlings. No *Pisolithus* sporophores were observed in these plots. It is not known at this time why basidiospores of *P. tinctorius* from the *Pisolithus* plots did not form more ectomycorrhizae on the *Thelephora* seedlings. Only 11 sporophores of *T. terrestris* were detected in all plots of seedlings with *Thelephora* ectomycorrhizae during the 3 years of this study.

During root evaluation it was observed that the fertilizer tablets were barely recognizable after the 2nd year. By the end of the 3rd year

Table 3. Survival and Growth of Loblolly Pine Seedlings with Pisolithus or Thelephora Ectomycorrhizae After 1, 2, 3, and 4 Years on a Coal Spoil in Virginia

End of Year	Treatment	Percent Survival	Height (m)	Root Collar Dia (cm)	Seedling Vol (cm^3)*
1	*Pisolithus*	95a	0.27a	0.7a	0.02a
	Thelephora	86b	0.23a	0.6a	0.01a
2	*Pisolithus*	92a	1.01a	3.1a	10.1a
	Thelephora	79b	0.81b	2.2b	4.4a
3	*Pisolithus*	81a**	2.00a	5.6a	64.5a
	Thelephora	68b**	1.54b	4.0b	26.6b
4	*Pisolithus*	81a**	2.99a	7.6a	173.8a
	Thelephora	67b**	2.14b	5.3b	61.8b

* Seedling volume=(root collar diameter, cm)^2 × height, cm.
**Decrease in survival due in part to tree removal by vandals after second year.
Means within a given year and tree species, but between treatments with a common letter are not significantly different at P=0.05.

only remnants of the tablets were found. Roots and ectomycorrhizae growing within 1 cm of the tablets were occasionally discolored, but visually they appeared to be functional.

The different treatments had a significant effect on concentrations of N, Ca, S, Fe, Mn, and Al in seedling foliage (Table 2). Phosphorus (0.101 to 0.124%), K (0.5 to 0.62%), Mg (0.093 to 0.134%), and Zn (24.2 to 36.1 p/m) were not significantly affected by treatment. Loblolly pine seedlings with *Pisolithus* ectomycorrhizae planted without the fertilizer tablet had a significantly higher concentration of foliar N than seedlings of the other three loblolly pine treatments after the 2nd and 3rd growing seasons. Seedlings with *Thelephora* ectomycorrhizae generally had higher concentrations of S, Fe, Mn, and Al in foliage than seedlings with *Pisolithus* ectomycorrhizae regardless of the fertilizer treatment.

Shortleaf pine seedlings with *Thelephora* ectomycorrhizae planted with the fertilizer tablet had lower concentrations of foliar N after both years and more Fe after the 2nd year than seedlings in other treatments. Calcium content was not affected. Generally, seedlings with *Thelephora* ectomycorrhizae had higher concentrations of foliar S, Fe, Mn, and Al than seedlings with *Pisolithus* ectomycorrhizae regardless of the fertilizer treatment.

Virginia Coal Spoil

Analysis of height measurements obtained 1 month after planting showed that the loblolly pine seedlings with *Pisolithus* ectomycorrhizae (12.4 cm) were significantly taller (29 percent) than seedlings with *Thelephora* ectomycorrhizae (9.6 cm). Spoil analysis revealed a pH of 3.4, organic matter of 1.5 percent, and less than 5 p/m of nitrate-N.

Pisolithus ectomycorrhizae significantly decreased mortality of seedlings after the 1st year, and increased all parameters of seedling growth by the 2nd year (Table 3). Seedling volumes were 129, 143, and 181 percent greater for seedlings with *Pisolithus* ectomycorrhizae than those with *Thelephora* ectomycorrhizae at the end of years 2, 3, and 4 respectively. *Pisolithus* seedlings which were initially 3 cm or 29 percent taller than those with *Thelephora* ectomycorrhizae were 85 cm or 40 percent taller by the end of the 4th year.

Seedlings in this study were also damaged by the winter of 1976-77 and had identical needle symptoms as seedlings on the Kentucky spoil. However, unlike those on the Kentucky spoil where all seedlings showed the same amount of needle burn in early March, the degree of injury on seedlings on the Virginia spoil varied by treatment. Seedlings with *Thelephora* ectomycorrhizae incurred 25 percent needle burn, but only about 5 percent of the needles were burned on seedlings with *Pisolithus* ectomycorrhizae. The more severe needle burn on *Thelephora* seedlings which reduced growth during the following year probably accounted for some of the growth differences between these seedlings and those with *Pisolithus* ectomycor-

rhizae. Seedlings with *Pisolithus* ectomycorrhizae had darker green foliage, longer needle length and more dense foliage than seedlings with *Thelephora* ectomycorrhizae during the entire 4 years.

The results of root examination of seedlings in this test were similar to those on the Kentucky coal spoil. Hyphal strands and ectomycorrhizae of *Pisolithus* dominated roots of seedlings in the *Pisolithus* treatment, whereas only *Thelephora* ectomycorrhizae occurred erratically on seedlings in the *Thelephora* treatment. A few *Pisolithus* ectomycorrhizae were observed on the *Thelephora* seedlings after the 2nd season. Sporophore production by *Pisolithus* was prolific in the *Pisolithus* plots. During the fall of 1977, over 200 sporophores of *Pisolithus* were observed in the six *Pisolithus* plots (1.1 sporophore/m^2) and 16 in the *Thelephora* plots. Sporophore size and shape were similar to those observed on the Kentucky spoil. *Thelephora* sporophores occurred occasionally in the *Thelephora* plots.

DISCUSSION

There is no doubt that *Pisolithus* ectomycorrhizae improved the survival and growth of loblolly and shortleaf pine seedlings on acid coal spoils. Seedlings with this ectomycorrhizal fungus had a root system that was physiologically capable of tolerating the adverse soil conditions and increasing absorption of essential nutrients from low concentrations. Due to a better physiological condition, these seedlings also exhibited fewer symptoms and recovered more rapidly from severe winter injury. Seedlings with *Thelephora* ectomycorrhizae not only grew more slowly, but they also accumulated higher levels of S, Fe, Al, and Mn in their needles than did seedlings with *Pisolithus* ectomycorrhizae. The significance of accumulation of these elements in needle tissue is not understood at this time, but high concentrations may reflect a physiological condition related to poor overall growth.

The fertilizer tablet improved growth of both pine species on the Kentucky coal spoil for the 1st year, but this effect did not continue through the 3rd year for loblolly pine. In fact, loblolly pine seedlings with *Pisolithus* ectomycorrhizae without fertilizer were as large as those with the fertilizer after 3 years. The gradual decrease in the effects of the fertilizer on loblolly pine was also detected in the needle analysis. Generally, after 3 years needles of seedlings with the fertilizer tablet were not as green as those without the tablet. Nitrogen concentrations in needles were correlated with needle color, i.e., slightly chlorotic needles had less N than those with a darker green color. Apparently the soluble nutrients in the tablets were readily available and stimulated growth for only the 1st or 2nd growing season. Afterwards, nitrogen and perhaps other nutrients became limiting and the seedlings developed symptoms of N-deficiency. The N-deficiency was more striking in seedlings with *Thelephora* ectomycorrhizae than in those with *Pisolithus* ectomycorrhizae.

The influence of the fertilizer tablet on growth of shortleaf pine lasted the entire 3 years of this test. Regardless of the ectomycorrhizal condition, seedlings with the tablet were larger than those without the tablet. However, some needle chlorosis was observed on seedlings planted with the tablet at the end of the 3rd year. This was correlated with a decrease in foliar N, especially in seedlings with *Thelephora* ectomycorrhizae. The deeper rooting habit of shortleaf pine, as compared to loblolly pine, may account for the more prolonged response of shortleaf pine to the fertilizer tablet. Nutrients, leached from the tablet moved downward through the spoil, and were utilized more effectively by the deep roots of shortleaf pine. Perhaps, more slowly available forms of nutrients, such as those in sewage sludge (Berry, In press) would be more advantageous for long term benefits to seedling growth on acid coal spoils.

It can be concluded that survival and growth of pine seedlings on acid coal spoils were significantly improved by ectomycorrhizae formed by specific fungi ecologically adapted to these spoils. In these studies, *Pisolithus tinctorius* spread rapidly to newly formed roots, grew extensively throughout the spoil, and reproduced in abundance. In contrast to this is *Thelephora terrestris* which did not spread rapidly to newly formed roots or reproduce well on the spoil. *Thelephora* obviously was not well adapted to conditions inherent on acid coal spoils because seedlings that initially had abundant *Thelephora* ectomycorrhizae at planting eventually suffered from a low incidence of functional ectomycorrhizae shortly after planting. Other ectomycorrhizal fungi, such as *Scleroderma aurantium*, *Amanita rubescens* and others (Schramm, 1966; Medve et al., 1977), have been reported to occur frequently on acid coal spoils. These fungi should be tested under field conditions, such as reported herein, to ascertain their adaptability to coal spoils and their effect on seedling growth.

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